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IS 13005 (1991): Method for detection of facultative pathogenic micro-organisms of bull semen [FAD 5: Livestock Feeds, Equipment and Systems]



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IS 13005 : 1991

Indian Standard

(Reaffirmed 2000)

DETECTION OF FACULTATIVE PATHOGENIC MICRO-ORGANISMS OF BULL SEMEN— METHOD

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FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Animal Reproduction Equipment and Systems Sectional Committee had been approved by the Food and Agriculture Division Council.

The quality of semen used for artificial insemination is of utmost importance for the success of the livestock improvement programme. This necessitates the semen to be free from various micro-organisms which would otherwise bring down the chances of successful insemination. The method for the detection of facultative pathogenic micro-organisms of bull semen has been enumerated in this standard.

In the preparation of this standard, assistance has been derived from ISO/TC 34/WG 5 N 72 'Bull sperm — Detection of facultative pathogenic micro-organisms' issued by International Organization for Standardization.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

Indian Standard

DETECTION OF FACULTATIVE PATHOGENIC MICRO-ORGANISMS OF BULL SEMEN — METHOD

1 SCOPE

1.1 This standard covers method for detection of facultative pathogenic micro-organisms of bull semen.

2 TERMINOLOGY

For the purpose of this standard, the following definition shall apply.

2.1 Facultative Pathogens

All the micro-organisms that cause, under certain predisposing conditions, local or general infection and disease, and induce antibody production detectable by immunologic methods such as serologic test.

NOTE—The main species of such organisms are—*Corynebacterium pyogenes*, *Pseudomonas aeruginosa*, haemolytic *Streptococcus* spp., *Escherichia coli*, *Haemophilus* spp., *Staphylococcus* spp., *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Mycoplasma agalactiae* subsp. *bovis* and pathogenic fungi including *Candida* spp., especially *Candida guilliermondii*, *Aspergillus* spp., *Mucor* and *Rhizopus* spp.

3 PRINCIPLE

3.1 Sterile assembling of semen from each bull arrived at a station for artificial insemination during its period of guarantee, that is, within 90 days, in general:

- a) Sterile assembling of semen and taking of blood sample for serum from each bull showing disorder in semen production (for example, its semen cannot be frozen three times consecutively).

- b) Thawing of deep-frozen semen, if need be.

3.2 Preparation of specified culture media suitable for detection and identification of facultative pathogens.

3.3 Inoculation of semen sample onto culture media and incubation for 48 to 72 h, as appropriate.

3.4 Identification of pathogens.

3.5 The pathogenic organisms isolated from semen and the serum taken from the same bull are subject to serological test.

4 COLLECTION OF SAMPLES

4.1 Obtaining Fresh Semen

The bull, the phantom (living or non-living) shall be thoroughly washed and the semen collecting set (artificial vagina, glass vessel or plastic bag) sterilized. One or more ejaculates should be obtained from the bull and handled in a sterile environment.

4.2 Taking of Deep-Frozen Semen

Take out randomly the necessary number of straws to make a sample of 1.5 ml of frozen semen from the containers of the respective production numbers.

4.3 Collecting Blood

Blood should be taken with sterile 'human blood collecting set' then put into dry glass tube of 15 mm × 100 mm. After keeping it at room temperature for half an hour, clotted blood should be loosened by having a sterile needle gone round the inner wall of the tube and put it in an incubator at 37°C for 1 h. The serum so released should be separated and transferred into a sterile glass tube of 10 mm × 100 mm.

5 APPARATUS

5.1 Besides usual microbiological laboratory equipment following shall be used.

5.1.1 *Oven* — for dry sterilization.

5.1.2 *Autoclave* — for wet sterilization.

5.1.3 *Incubator* — able to keep $37 \pm 1^\circ\text{C}$.

5.1.4 *Glass Tubes* — 15 mm × 100 mm and 10 mm × 100 mm.

5.1.5 *Petridishes* — made from glass or plastics of diameter 90 to 100 mm.

5.1.6 *Volumetric Pipette* — of 1 ml capacity, calibrated for each 0.1 ml.

5.1.7 *Electric pH Meter*

6 CULTURE MEDIA AND DILUTION FLUID

6.1 Basic Materials

6.1.1 It is recommended that dehydrated basic components or completely dehydrated media should be used for the preparation of culture media.

6.1.2 The chemicals used shall be of analytical grade.

6.1.3 The water used shall be double distilled and deionized.

6.1.4 If the culture media and the dilution fluid are not used immediately, they shall be kept in dark at a temperature between 0 and 5°C and under conditions that prevent any change in composition.

6.2 Dilution Fluid

Use physiological saline solution, with NaCl content of 0.85 percent.

6.3 Specific Culture Media

- a) Solid plate agar media should be applied.
- b) *Preparation of Culture Media*— Dissolve by boiling the components or the dehydrated complete medium in water. Adjust pH, if necessary (checking with pH meter), so that it is 7.0 ± 0.2 at 25°C after sterilization. Dispense the medium into bottles in 500 ml quantities that level approximately half the volume of the container. Sterilize them in an autoclave at $121 \pm 1^\circ\text{C}$ for 20 min. If the medium is not used immediately, put it into a refrigerator at 4°C.

6.4 Water-Agar Medium

7 PREPARATION OF TEST SAMPLE

7.1 A test sample of 0.1 ml of semen shall be taken from the middle of mixed ejaculate(s) with sterile pipette, transferred into 10 ml of sterile physiological saline solution and thoroughly mixed (dilution 1 : 100). Then make second dilution 1 : 1 000 from the first one. Both dilutions should be put into a refrigerator at 4°C until their turn is due either immediately or within a maximum of 2 h.

7.2 The deep-frozen semen shall be stored in liquid nitrogen until bacteriological examination. For sampling purposes, it should be taken from the large storing container and transferred to a small laboratory container.

7.2.1 Semen in any type of packaging should

be thawed by keeping it in water bath at $37 \pm 0.5^\circ\text{C}$ for 3 minutes.

7.2.2 If the deep-frozen semen contains antibiotics, it should be leached by centrifuging (3 600 rev./min for 20 min) three times its suspension in sterile physiological saline solution (0.85 percent NaCl). Use 3-4 ml saline solution each time. After the third treatment discard the supernatant and only 1 ml saline solution shall be added to the sediment. Starting from this, 1 : 100 and 1 : 1 000 dilutions should be made.

7.2.3 Before dilution, fresh or deep-frozen semen after thawing shall be kept in refrigerator at 4°C, but only for maximum of 1 hour.

8 PROCEDURE

8.1 Inoculation

Before inoculation all the media poured previously into sterile petridishes should be put into the incubator at $37 \pm 1^\circ\text{C}$ for 15 min. Inoculations should be carried out in the same manner for either fresh or freshly thawed semen. 0.1 ml of semen diluted to 1 : 100 should be transferred by means of sterile pipettes on the surface of each specific medium and smeared well with a sterile glass rod. Take the semen dilution of 1 : 1 000 and another set of the specific media and inoculate each of them in the same way, using new sterile pipettes and glass sticks.

8.2 Incubation

Place the inoculated petridishes upside down in the incubator at $37 \pm 1^\circ\text{C}$ for 48 to 72 h.

8.3 Additional Culturing

After the specified period of incubation, examine the cultures and make further inoculations from them if pure cultures are to be obtained.

8.4 Antibiotic Resistance Test

Test pure bacterial culture for its resistance to antibiotics in accordance with the disc or the dilution method.

8.5 Serological Test

The constant suspension of pure bacterial culture and the gradually diluted serum of the suspected animal should be examined by agglutination test.

9 CONCLUSION FROM RESULTS

9.1 After obtaining pure cultures, identify the pathogens so isolated.

9.2 When the micro-organisms listed in **2.1** (Note) have been identified from the *fresh semen* and significant antibody titer (above 1:40) was found in the serum of the infected animal, the bull shall be declared carrier of pathogens. The resistance of pathogens against antibiotics should also be determined.

9.3 When *deep-frozen semen* in pure culture was found to be infected with the micro-organisms mentioned in **2.1** (Note) further use (for

example export) of this production Number of the semen shall be forbidden.

10 TEST REPORT

10.1 The test report shall show the species of the pathogens or at least its genus, and its resistance against antibiotics. It shall also mention all circumstances that may have influenced the result. The report shall include all details required for complete identification of the sample of semen or blood.

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